#24
6-22-02

IN THE UNITED STATES ~~PATENT AND~~ TRADEMARK OFFICE

APPLICANT: Rosenblum, M. G.	§	**ART UNIT:**
	§	**1642**
FILED: February 19, 1998	§	
	§	
SERIAL NO.: 09/026,882	§	**EXAMINER:**
	§	**S. Huff**
	§	
FOR: Immunotoxins Directed Against CD33 Related Surface Antigens	§	**DOCKET: 5442C/CIP**

The Honorable Commissioner of Patents and Trademarks
BOX AF
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, Michael G. Rosenblum, do hereby depose and say as follows:

1. I have read U.S. Patent Application Serial No. 09/026,882, filed on February 19, 1998, and I am aware of the contents of this application, including all Office Actions and prior art cited against this application.

2. I am an inventor of the claimed subject matter of this application. I am skilled in the areas of immunopharmacology and molecular biology as evidenced by that fact that I am a Professor and the Chief of the Section of Immunopharmacology and

Targeted Therapy, Department of Bioimmunotherapy at the University of Texas, M.D. Anderson Cancer Center in Houston, Texas.

3. An issue related to the patentability of the claimed products and methods is that the combination of cited prior art render the products and methods claimed obvious to one of ordinary skill in the art. The following experiments were conducted to provide evidence of the non-obviousness of the claimed subject matter.

4. Human M195 antibody conjugated to recombinant gelonin (HuM195/rGel) was used in an *in vitro* study of bone marrow purging in acute myeloid leukemia. Peripheral blood progenitor cells from leukemic patients receiving autologous stem cell implantation were mixed with acute myeloid leukemia HL60 cells to simulate a remission in bone marrow contaminated with about 10% leukemic cells. These cells were clonogenically cultured and treated with 1-10 nM of HuM195/rGel with or without the freeze/thaw procedure. The freeze/thaw procedure involves the freezing of cells in cryotubes for 24 hours, and quickly thawing these cells in a 37^{0}C water bath before incubation with culture medium. Such a procedure has been reported to be cytotoxic to cells and to

work synergistically with HuM195rGel. A similar experiment was performed with by treating cultured chronic myeloid leukemic cells with HuM195/rGel.

5. Attached to this Declaration are Figures 1-4. Figure 1 compares the number of colony formations in peripheral blood progenitor cells treated with HuM195/rGel with or without the freeze/thaw procedure. Figure 1 shows a significant reduction in colonies after synergistic treatment of HuM195/rGel with freeze/thaw procedure. Figure 2 shows the time-dependent recovery of colonies after treatment with HuM195/rGel. Figure 2 observes a percent drop in colony recovery with increasing concentration of HuM195/rGel. Figure 3 shows a reduction in colony formation with increasing concentration of HuM195/rGel as well the synergistic effect of HuM195/rGel with the freeze/thaw procedure. Figure 4 shows the simulated bone marrow remission mixture of peripheral blood progenitor cells with HL60 cells treated with HuM195/rGel with or without the freeze/thaw procedure. Figure 4 shows the significant reduction in colonies after HuM195/rGel treatment and a greater reduction in colony formation after synergistic treatment of HuM195/rGel with the freeze/thaw procedure.

6. These experiments demonstrate that HuM195/rGel was not cytotoxic to peripheral blood progenitor cells. The inhibition rates did not differ between cells treated HuM195/rGel and cells treated with HuM195/rGel and the freeze/thaw procedure, suggesting that any toxicity is observed is attributable to the freeze/thaw procedure.

7. HuM195/rGel was also used in a phase I clinical study where HuM195/rGel was administered to patients with myeloid malignancies. During the experiment, dose levels of 10, 12, 18 and 28 mg/m^2 of HuM195/rGel were administered at a regimen of 1 hour infusion every 72 hours over 2 weeks. The 17 patients in this clinical trial experienced no toxicity even at the 28 mg/m^2 dose range and only 2 of the 17 patients (about 12%) manifested human anti-gelonin antibody responses, instead of the expected 80% toxicity. This low incidence of human anti-gelonin antibody response was shown not to be dose related. One patient treated at the 28 mg/m^2 dose level showed a 50% decrease in leukemic content during a Day 14 biopsy. No exhibition of antigenicity was observed in this patient.

8. Further attached to this Declaration are Figure 5 and Table I. Figure 5 illustrates the pharmacokinetics of HuM195/rGel. The shaded rectangle reflects the effective range of blood level HuM195/rGel of between 200–400 ng/ml. Table I shows the lack of antigenicity observed in patients treated with varying doses of HuM195/rGel. Only 2 of the 17 patients exhibited antigenicity at a 1:100,00 dilution of HuM195/rGel but antigenicity was not dose related.

9. I hereby declare that all statements herein of my own knowledge are true, that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 5/15/02

Dr. Michael G. Rosenblum

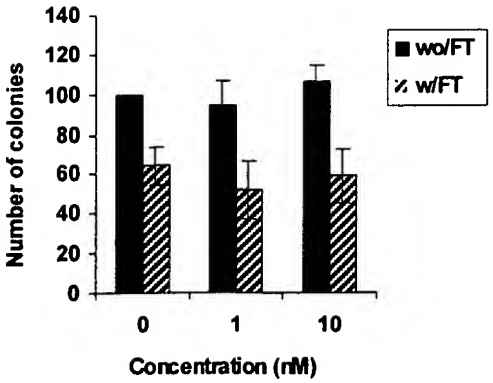

Figure 1. Number of colonies formed by PBPCs treated with HuM195/rGel without (wo) or with (w) the F/T procedure. Each bar represents a median of 9 normal donor samples and includes standard error bars.

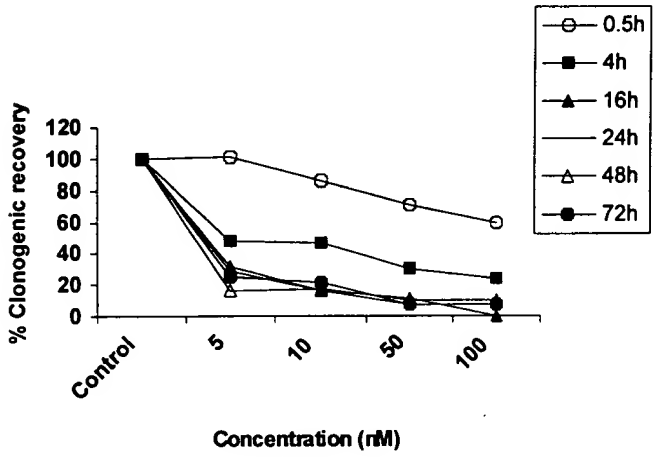

Figure 2. Time-course studies to determine the optimum time point and concentration of HuM195/rGel that provides maximum toxicity on HL60 cells.

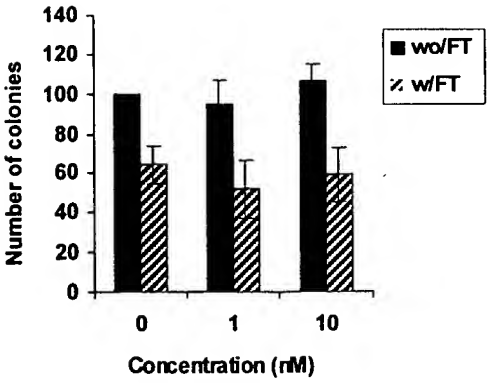

Figure 1. Number of colonies formed by PBPCs treated with HuM195/rGel without (wo) or with (w) the F/T procedure. Each bar represents a median of 9 normal donor samples and includes standard error bars.

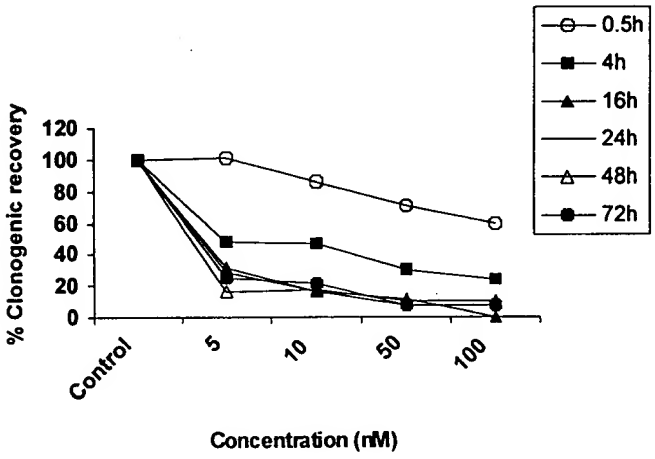

Figure 2. Time-course studies to determine the optimum time point and concentration of HuM195/rGel that provides maximum toxicity on HL60 cells.

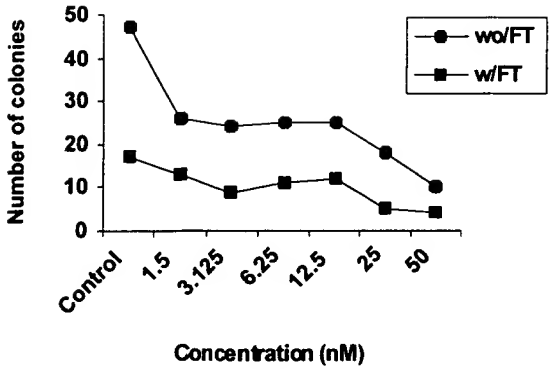

Figure 3. Dose-response curve for a patient with accelerated-phase CML without (wo) or with (w) the F/T procedure.

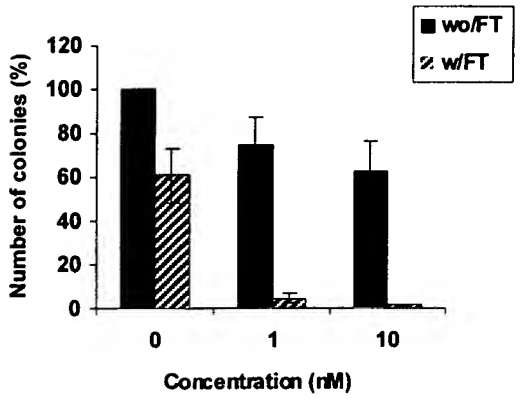

Figure 4. Number of colonies formed by mixture of PBPCs and HL60 cells (at a 9:1 ratio) treated with HuM195/rGel without (wo) or with (w) the F/T procedure. Each bar represents a median (with standard error bars) of 9 normal donor samples mixed with HL60 cells. Colonies are formed only by HL60 cells.

Pharmacokinetics of HuM195/rGel

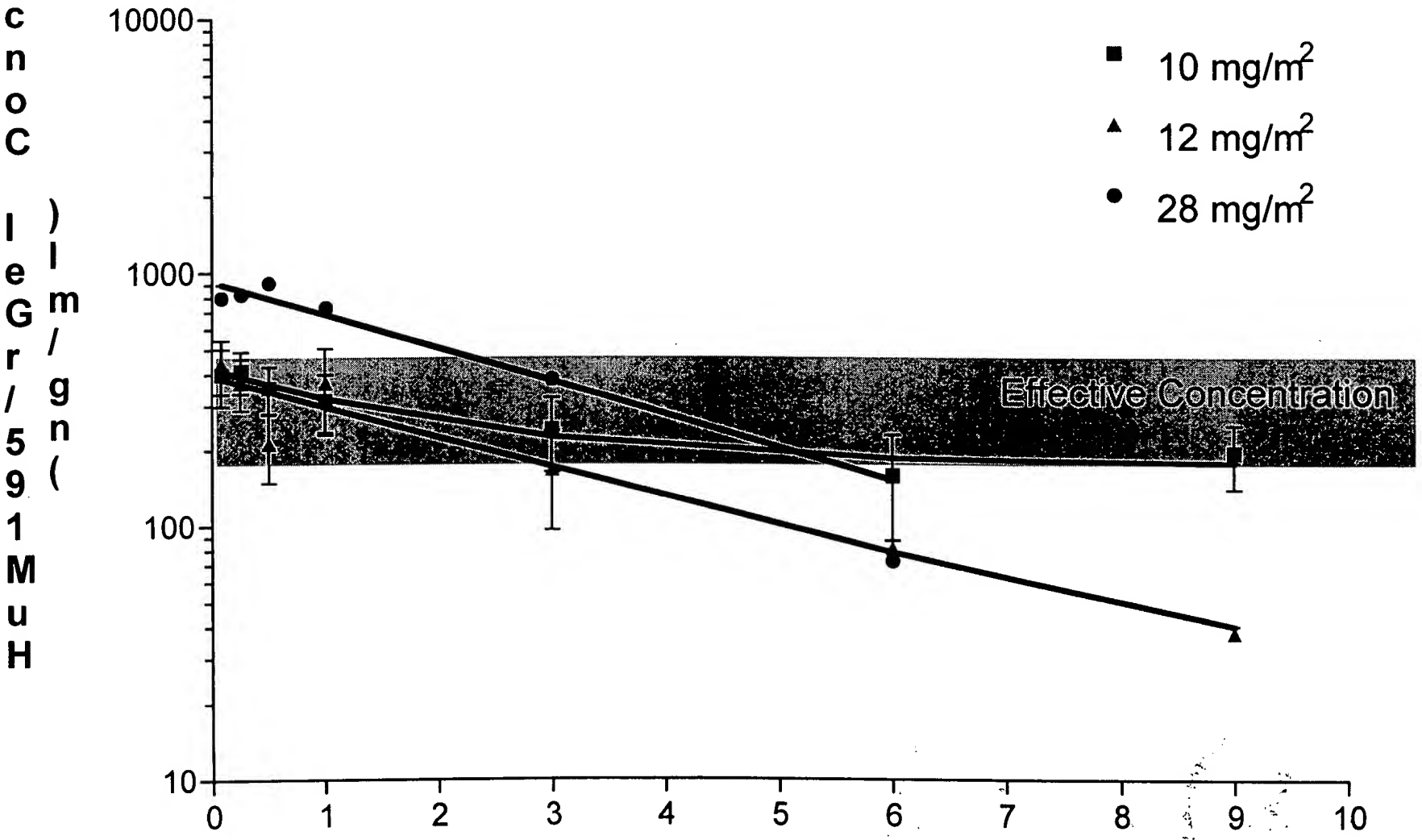

Figure 5

Table I. Antigenicity of HuM195/rGel

Dose:	Patient #	Pre-Dose	Dose 4
10 mg	1	-	-
	2	-	-
	3	-	-
	4	-	-
	5	-	-
	6	-	-
12 mg	7	-	-
	8	-	-
	9	-	+(1:100,000)
	10	-	-
	11	-	-
	12	-	-
18 mg	13	-	-
	14	-	-
	15	-	+(1:100,000)
28 mg	16	-	-
	17	-	-